REMARKS

Claims 19, 21-29 and 42-44 are currently pending in the application. Claims 1-18, 20 and 30-41 relating to a non-elected invention are canceled. Claims 43 and 44 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Rejection of claims 43 and 44 under 35 U.S.C. §112, second paragraph

Claim 43 is rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness. Applicants have amended step (b) of claim 43 to refer to "concanavalin A adherent and non-adherent cells". Applicants have also amended step (c) of claim 43 to refer to "said non-adherent cells from step (b)". Support for this amendment is found in the specification at page 26, lines 7-14 and in Example 1 at page 39.

Claim 44 is rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness. Applicants have amended claim 44 to delete the term "human".

In view of all of the above, Applicants respectfully request withdrawal and reconsideration of the 35 U.S.C. §112, second paragraph rejections of claims 43 and 44.

Rejection of claims 24-29 and 44 under 35 U.S.C. §102(b)

Claims 24-29 and 44 are rejected under 35 U.S.C. §102(b) for alleged lack of novelty in view of WO 97/15310.

Applicants respectfully traverse the rejection.

The Examiner states at page 5, "Peck et al. teaches isolated pancreatic stem cells, including isolated human pancreatic stem cells, wherein the pancreatic stem cells can be induced to differentiate into mature cell types such as insulin-producing beta cells, glucagon producing alpha cells, and are further capable of producing islet like aggregates comprising different mature cell types ...While Peck et al. does teach methods for identifying marker proteins specific to pancreatic stem cells versus more differentiated cell types,... and also teaches isolating

pancreatic stem cells based on specific marker proteins...Peck et al. does not specifically teach that nestin is a marker for pancreatic stem cells. However, since Peck et al. teaches pancreatic stem cells and further teaches that these cells have the same capacity to differentiate into more mature cell types as nestin positive pancreatic stem cells disclosed in the instant specification, nestin expression by pancreatic stem cells is considered an inherent property of the stem cells disclosed by Peck et al."

Claims 24-29 and 44 include the limitation of "an **isolated, nestin-positive** pancreatic or liver stem cell that is not a neural stem cell." (Emphasis added)

The WO '310 application does not teach "an **isolated, nestin-positive** pancreatic or liver stem cell that is not a neural stem cell" as required by claims 24-29 and 44 of the instant application. (Emphasis added)

Applicants submit that the Examiner must provide rationale or evidence tending to show inherency to properly make a rejection under 35 U.S.C. 102 when the prior art is silent as to an inherent characteristic (MPEP §2112).

A "stem cell" is defined in the instant application at page 7, line 19-21 as "a undifferentiated cell which is capable of essentially unlimited propagation either *in vivo* or *ex vivo* and capable of differentiation to other cell types."

A "pancreatic stem cell" is defined in the instant application at page 10, lines 9-12 as "a stem cell that has been isolated from pancreatic tissue and/or a cell that has all of the characteristics of: nestin-positive staining, nestin gene expression, cytokeratin-19 negative staining, long-term proliferation in culture, and the ability to differentiate into pseudo-islets in culture."

"Isolated" is defined in the instant application at page 11, lines 3-15 as follows:

> "Isolating" a stem cell refers to the process of removing a stem cell from a tissue sample and separating away other cells which are not stem cells of the tissue. An isolated stem cell will be generally free from contamination by other cell types and will generally have the capability of propagation and differentiation to produce mature cells of the tissue from which it was isolated. However, when dealing with a collection of stem cells, *e.g.*, a culture of stem cells, it is

understood that it is practically impossible to obtain a collection of stem cells which is 100% pure. Therefore, an isolated stem cell can exist in the presence of a small fraction of other cell types which do not interfere with the utilization of the stem cell for analysis or production of other, differentiated cell types. Isolated stem cells will generally be at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 99% pure. Preferably, isolated stem cells according to the invention will be at least 98% or at least 99% pure."

The instant specification teaches one embodiment of a method of isolating a nestin-positive human pancreatic stem cell in Example 1 (see page 39) wherein the instant specification states:

"Rat islets were isolated from the pancreata of 2-3 month old Sprague-Dawley rats using the collagenase digestion method described by Lacy and Kostianovsky. Human islets were provided by the Diabetes Research Institute, Miami, FL using collagenase digestion. The islets were cultured for 96 hrs at 37°C in 12-well plates (Falcon 3043 plates, Becton Dickinson, Lincoln Park, NJ) that had been coated with concanavalin A. The culture medium was RPMI 1640 supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, 10mM HEPES buffer, 100 μg/ml streptomycin, 100 units/ml penicillin, 0.25 μg/ml amphotericin B (GIBCO BRL, Life Science Technology, Gaithersburg, MD), and 71.5 mM β-mercaptoethanol (Sigma, St. Louis, MO).

After 96 hrs, fibroblasts and other non-islet cells had adhered to the surface of concanavalin A coated wells and the islets remained floating (did not adhere to the surface). At this time, the media containing the islets were removed, centrifuged down, and the purged islets replated in 12-well plates without a coating of concanavalin A. The islets were then cultured in the above RPMI 1640 medium supplemented with 20 ng/ml of basic fibroblast growth factor-2 and 20 ng/ml of epidermal growth factor.

The islets adhered to the surface of the plates, and cells grew out and away from the islets in a monolayer. **These cells that form a monolayer were nestin-positive by immunostaining with a rabbit anti-rat nestin antiserum** developed by Dr. Mario Vallejo at the Massachusetts General Hospital. Other nestin antibodies may be used, for example the R.401 antibody described hereinabove, or the MAB533 antibody. A monoclonal antibody specific for rat embryo spinal cord nestin, MAB353, ATCC No. 1023889; is described in Journal of Neuroscience 1996; 16:1901-100; and also available from Chemicon International, Single Oak Dr., Temecula, CA 92590 USA. **After two weeks of culture, several (3-5) of the nestin-positive monolayer cells were removed by picking with a capillary tube (cylinder cloning) and were replated** on the 12-well plates (not coated with concanavalin A) and cultured in the RPMI 1640 medium further supplemented with bFGF-2 and EGF. The cells propagated at a rapid rate and reached confluence after six days of culture. After 12 days of culture, the cell monolayer formed waves in which they begin to pile up in a co-

> linear manner. On day 15 of culture, the cell waves began to condense, migrate into spheroid bodies and by day 17 the surface of the wells contained these spheroid bodies (ca. 100 μm in diameter), empty spaces, and a few areas of remaining monolayer cells. Several of these monolayer cells were re-picked and re-cloned and the process described above occurred again in precisely the same temporal sequence." (Emphasis added)

Additional embodiments of methods of isolating a nestin-positive human pancreatic stem cells are discussed in the attached Rule 1.132 declaration of Dr. Habener.

The invention of the WO '310 application "concerns the discovery that functional islets containing insulin-producing β-cells, as well as other islet cell types, can be grown in long-term cultures from pluripotent stem cells, which give rise to islet producing stem cells, IPSCs" (see page 8, lines 27-30). The WO '310 application discloses at page 12, lines 21-23 that "IPSCs are a small population of cells derived from ductal epithelial cells (i.e., these cells are pancreas-derived but are not differentiated islet cells)".

The WO '310 application teaches a method of growing IPSCs at page 13 line 29 through page 14, line 25 wherein it is stated that,

> " [t]he method of the subject invention involves making suspensions of cells, including stem cells, from the pancreas of a mammal. . .The cell suspensions are prepared using standard techniques. The cell suspension is then cultured in a nutrient medium that facilitates the growth of the IPSCs, while at the same time severely compromising the sustained growth of the differentiated or mature cells other than IPSCs. . . What is required for such media is that they have little or no glucose (less than about 1 mM) and low serum (less than about 0.5%). The high amino acid concentrations are preferably of amino acids known to be essential for the cells of the species being cultured, and provide a carbon source for the cultured cells. In addition, at least one rudimentary lipid precursor, preferably pyruvate, is provided. These conditions are so stressful to most differentiated cell types that they do not survive. Surprisingly, however, upon extended culture of cells from pancreatic tissue without re-feeding (about 3 weeks) IPSCs do survive and after extended culture, begin to proliferate."

The WO '310 application discloses a method of growing IPSCs at pages 22-24. However, the method of growing IPSCs presented in the WO '310 application (see pages 13-14 and 22-24) does not include a step wherein nestin-positive pancreatic stem cells are isolated. The WO '310 application therefore teaches "suspensions of cells, including stem cells, from pancreas of a

mammal" (page 13, lines 29-30) and a method of growing islet producing stem cells (IPSCs) but does not teach an **isolated nestin-positive** pancreatic stem cell that is not a neural stem cell, as required by claims 24-29 and 44 of the instant application.

The WO'310 application does not disclose the percent purity of the IPSCs that are grown according to the disclosed method. The attached Rule 1.132 declaration of Dr. Habener asserts that the percentage of nestin-positive cells in the pancreas is believed to be in the range of 0.2 to 5 %. Given the very low percentage of nestin-positive stem cells in the pancreas, one of skill in the art would not accept or predict that the method of growing stem cells presented in the WO '310 application would result in an **isolated** nestin-positive pancreatic stem cell as required by claims 24-29 and 44 of the instant application, since this method lacks a step wherein nestin-positive stem cells are isolated. In view of the above, Applicants respectfully assert that one of skill in the art would not accept or predict that the suspension of stem cells grown according to the method described in the WO '310 application is an isolated nestin-positive pancreatic stem cell as required by instant claims 24-29 and 44, and as defined in the instant application.

The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) ...; *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. **Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.**' " *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (Emphasis) (MPEP §2112, citations omitted).

In view of the above, one of skill in the art would not accept that "an isolated, nestin-positive human pancreatic or liver stem cell that is not a neural cell" as required by claims 24-29 and 44 of the instant application would inherently be identical to the suspension of stem cells grown according to the methods of the WO '310 application since, given the small percentage of nestin-positive cells in the pancreas, and due to the absence of a step wherein nestin-positive

pancreatic stem cells are isolated, it is unlikely that the cells of the WO '310 application are **isolated** nestin-positive pancreatic stem cell as defined by the instant application (that is, at least 30% pure).

One of skill in the art would also not accept that it is necessarily probable or possible that the suspension of stem cells grown according to the methods of the WO '310 application are inherently "an isolated, nestin-positive human pancreatic or liver stem cell that is not a neural stem cell" as required by claims 24-29 and 44 of the instant application.

Applicants respectfully submit that the Examiner has not provided extrinsic evidence that makes clear that "the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill". That is, the Examiner's assertion that "the cell population taught by WO '310 is identical to that claimed in the instant application" is based on mere probabilities and possibilities.

In view of all of the above, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 24-29 and 44.

Provisional Rejection of claims 24-28 under 35 U.S.C. §101

Claims 24-28 are provisionally rejected under 35 U.S.C. §101 for allegedly claiming the same invention as that of claims 39 and 43 of copending Application No. 09/963,875.

Applicants submit that claims 39 and 43 of copending Application No. 09/963,875 will be amended thereby rendering the provisional rejection moot.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the above rejection.

Provisional Double Patenting Rejection of Claims 19, 21-23, 29 and 43-44

Claims 19, 21-23, 29 and 43-44 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 39-43 of copending Application No. 09/962,875.

In response to this rejection, Applicants submit that they will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would

extend beyond the term of a patent issuing from the 09/962,875 applications, upon notification of allowable claims in the present application.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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